

Kogenate/Kogenate FS

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Inhibitor formation with Kogenate®

- Description of the molecule
- Preclinical testing
- Neoantigenicity studies
- Prospective trials (PTPs and PUPs)
 - Kogenate
 - Kogenate FS
 - Post marketing studies
- Spontaneous reports to the manufacturer



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Kogenate and Kogenate FS are full length recombinant FVIII products

- Kogenate and Kogenate FS produced by identical cell line and fermentation procedure
- Derived from full length FVIII cDNA
- Expressed in BHK cells without co-expression of vWF
- Continuous perfusion fermentation >100 days in culture
- Immunoaffinity purified (MoAB against the light chain); same for both products
- Thrombin cleavage sites same as PD FVIII--Western blotting identical band patterns with PD FVIII
- N- and C-terminal sequence analysis concur with cDNA sequence
- Trypsin digest /reverse phase HPCL similar to PDFVIII
- Carbohydrate composition similar to PD-FVIII (high mannose type, bi-, tri-, tetra-antennary complex type sugar chains)*
 - presence of blood group determinants in bi-antennary structures in PD FVIII--not present in rFVIII
 - presence of Gal α 1 \rightarrow 3Gal group in rFVIII
 - recovery studies in primates with antibody to this structure and in humans showed similar results with PD FVIII

Characterization of rFVIII

- Coagulation assay
- Kinetics of Fxa formation
- Inhibition studies
- Inactivation by APC
- Binding to VWF
- Western blot
- Amino acid analysis
- N-term, C-term sequence
- Peptide mapping
- SDS-PAGE
- Carbohydrate analysis

Hironaka, Furukawa, Esmon et al. JBC 267:8012, 1992



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Kogenate preclinical testing

- Preclinical package for Kogenate/KogenateFS
 - Acute (single dose) and subacute (limited by formation of heterologous antibodies) performed in mice, rats, rabbit, dogs and non-human primates
 - repeated doses up to 5
 - no issues observed at doses of 100-300 IU/kg as assessed by weight gain, blood chemistry, necropsy, histopathology
 - Subchronic/chronic/genotoxicity/reproductive toxicity studies were not performed due to expected immune response to heterologous FVIII protein
 - All excipients are GRAS

Kogenate neo-antigenicity study

- Neo-antigenicity study--Kogenate
 - Rabbits immunized with Kogenate (rFVIII) in FA
 - Hyperimmune sera adsorbed with PD-FVIII
 - Flow through activity against PD-FVIII by Western blot decreased in parallel with activity against r-FVIII
 - Positive control (BSA) remained reactive
 - Results confirmed in a competitive ELISA
 - Immunization with a B-domain partially deleted rFVIII showed continued reactivity in this assay after adsorption removed reactivity with PD FVIII
 - **Conclusion:** Kogenate antibodies cross-reactive with PD-FVIII and are depleted in the same manner as PD-FVIII suggesting no neo-antigens
- Neo-antigenicity study--Kogenate-FS
 - Rabbits immunized with Kogenate FS in FA
 - Hyperimmune sera adsorbed against Kogenate
 - Remaining antisera had no reactivity against Kogenate FS
 - **Conclusion:** Kogenate FS antibodies cross-react with Kogenate and suggest no neo-antigens

Esmon, Kuo and Fournel: Blood 76:1593. 1990



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Clinical studies



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Studies in previously treated patients (PTP)-Kogenate

KOGENATE PTP CLINICAL TRIALS

Study Number	Year of study	Clinical Phase	No. of Patients	No. of infusions	Follow up median	No. of patients developing new inhibitors
Bay 6240/581-8801	1988-1994	III	103 PTPs	17,338	4.6 years (338-2159)	1
BAY6240/0108	1992-1995	III	13 PTPs	982		1 infrequently-treated patient developed a low titer transient inhibitor (0.78BU)
BAY6240/0111	1993-1995	III	39 PTPs	3,679		None*
BAY6240/0115	1998	IIIB	20 PTPs	NA		None
Japan PTPs	1991	I	20 PTPs	1247		None
TW 581-9401	1999	III	10 PTPs	633		None
TOTAL			205	>23,879		2 (1 transient)

*One patient developed a positive inhibitor assay (0.72 BU) which could not be confirmed and was subsequently tested negative without modification in treatment: interpreted as a spurious laboratory finding.



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Studies in previously treated patients (PTP)-Kogenate FS

KOGENATE FS PTP CLINICAL TRIALS

Study Number	Year of study	Clinical Phase	No. of Patients and patient group	No. of infusions	Follow up	No. of patients developing new inhibitors
BAY14-2222/0101	1996-2001	I, II, III	34 PTPs	Stage I: 15 Stage II/III: 14,469	4.0 years (0.5-4.6)	None
Bay 14-2222/0102 100075/100109 /595-9601	1996-2000	I, II, III	39 PTPs	Stage I: 20 Stage II/III: 10,352	3.9 years (0.08-4.2)	None
BAY14-2222/0103 (Japan)	1999	III	20 PTPs	1,541		None
Bay 14-2222/ 100124	1998	II PK	21 PTPs	42		None
Bay 14-2222/ 100265	2000	II PK	21 PTPs	42		None
Bay14-2222/ 450008	2001	IV PK	17 PTPs	34		None
TOTAL			152	26,480		0



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Kogenate PUP study: one of the first to evaluate inhibitor formation prospectively

- Study design to measure inhibitors more frequently than typical practice (every 3 months)
- Brought to light the phenomenon of transient and low titer inhibitors that may have been undetected in previous retrospective case series
- Results from these studies on inhibitor formation were found to be consistent with reported development of inhibitors with PD-FVIII



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Studies in previously untreated patients (PUPs)-Kogenate

KOGENATE PUPS CLINICAL TRIALS

Study Number	Year of study	Clinical Phase	No. of Patients and patient group	No. of infusions	Follow up	No. of patients developing new inhibitors
BAY6240/0104/581-8804	1988-1997	III/IV	102 PUP	15,475	4.6 years (0.4-6.3)	21 (19/65 severe 29%) (12 high; 9 low <10 BU)*
BAY6240/0110	1997	III	9 PUP	991		3 (30%) (all low titer <5BU)
BAY6240/0112	2003	IV	27 PUP	1,152		8 (29.6%) (2 high; 6 low)
Japan PUPs	Pending	IV	47 PUP	1,496		15 (32%) unverified
TOTAL			185	19,114		47 (25.4%)

* median exposure days 9 (3,54)



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Studies in previously untreated patients (PUPs)-Kogenate FS

KOGENATE FS PUPS CLINICAL TRIALS

Study Number	Year of study	Clinical Phase	No. of Patients and patient group	No. of infusions	Follow up	No. of patients developing new inhibitors
Bay 14-2222/0104	1997-2001	III	31PUPs/MTP	4,696	2.4 0.3-3.07)	4 (all low titer)
Bay 14-2222/ 100074 /595-9701	1997-2001	III	30 PUP/MTP*	4,699	2.0-2.5 (0.02-3.01)	5 (all high titer)
TOTAL			61	9395		9 (60 evaluable—15%)

MTP=Minimally treated patients (≤ 4 exposure days)

*one patient (MTP) had pre-existing inhibitor at enrollment



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Post-marketing studies--Kogenate

KOGENATE POST-MARKETING STUDIES

Study Number	Year of research report	Clinical Phase	No. of Patients and patient group	No. of infusions	Follow up	No. of patients developing new inhibitors
Substudy of BAY6240/0104 (5 year safety and orthopedic follow-up)	2003	IV	38 PUPs	NA		None
Japan PTPs PMS	pending	IV	123 PTPs	5,618		5 * (unverified)
Continuous Infusion Study France	1999	IV	3	NA		None

* The 5 subjects who developed inhibitors (unmodified Bethesda assay) in this study were reported to have had >100 ED. In one case, it could not be determined whether the subject had a positive inhibitor assay in the past; the highest inhibitor titer in this subject was reported at 0.9 BU. Inhibitor titers for the remaining 4 cases were 0.51, 0.81, 2.1, 0.7.



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Post-marketing studies--Kogenate FS

KOGENATE FS POST-MARKETING STUDIES

Study Number	Year of research report	Clinical Phase	No. of Patients and patient group	No. of infusions	No. of patients developing new inhibitors.
PMS Europe BAY 11145	Study Ongoing	PMS	200 PTP/PUP	Unknown	None
PMS BAY 100317	Study Ongoing	PMS	45 PTP/PUP	Unknown	None
PMS Japan KG0201	Study Ongoing	PMS	310 PTP/PUP	Unknown	None



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Canadian studies on inhibitor formation following product switch

- Design:
 - 3 samples per patient, prior to conversion then twice following conversion
 - performed at central lab--both Bethesda and Nijmegen modification
- Study 1 (Giles et al. Trans Sci 1998)
 - Switch from PD to Kogenate
 - Inhibitor results similar at pre-conversion, year 1 and year 2 and similar to patients converted to high purity PD
- Study 2 2001- present (to be presented at ASH 2003)
 - Switch from Kogenate to Kogenate FS
 - Nijmegen-modified Bethesda assay
 - 354 were enrolled had subsequently received KG-FS: all 354 had a first sample (pre-switch), 221 had a second sample, and 116 have had a third sample
 - no new inhibitors detected



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Reports to Bayer Drug Safety



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Spontaneous reports of inhibitors to Bayer

- Inhibitors reported--total 58--34 possible PTPs
 - 2 confirmed to have a prior history
 - 22 PUPs or ≤ 20 EDs
 - **7** confirmed >20 -100 ED
 - **16** were unassessable given information provided
 - **8** possible PTP
 - **3** felt to represent true PTP
- 4,679 million units distributed 1994-2003
 - assume annual dose of 212,000 IU per adult (full prophylaxis)
 - highly conservative given significant use of recombinant FVIII in pediatric population and little penetrance of full prophylaxis in adults
 - ~22,000 patient years of exposure
 - **Rate = $34 / 22000$ pt yrs = 1.5 per 1000 pt yrs**
 - assume annual dose of 50,000 IU per adult (on demand 1 bleed per month)
 - ~ 94,000 patient years of exposure
 - **Rate = $34 / 94000 = 0.4$ per 1000 pt yrs**
- Compare baseline (Rosendaal) =
~4 per 1000 pt yrs

Rosendaal et al. Blood 81:2180, 1993



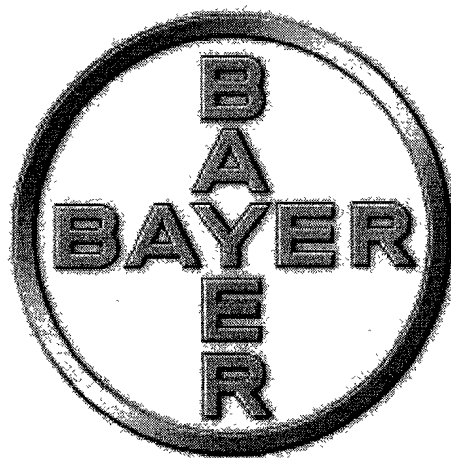
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Summary

- Over 15 years clinical experience with the Kogenate molecule
- 12 prospective studies in 342 PTPs--2 *de novo* inhibitors-1 low titer transient
- 5 prospective studies in 246 PUPs--inhibitor development as expected for a FVIII product
- 2 large post-licensing studies of switching from PD to Kogenate or Kogenate to Kogenate FS--no increase in inhibitors
- Spontaneous reporting of inhibitors at or below that observed in Dutch retrospective study
- Overall strong clinical evidence supporting safety regarding inhibitors with full length recombinant FVIII (Kogenate/Kogenate FS)



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Clinical trials Koate DVI-PTPs

KOATE DVI PTP CLINICAL STUDIES

Study Number	Year of study	Clinical Phase	No. of Patients and patient group	No. of infusions	Follow up	No. of patients developing new inhibitors
BAY-X9209 583-9501	1995-1996	III	20* PTP	1053	6 months	None
BAY-X9209 583-9501	1997	II	15** PTP	972	6 months	None
TOTAL			35	2025		0

*12/19 patients followed for 6 months were HIV positive

**all patients were HIV negative



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Spontaneous reports of inhibitor formation with Koate DVI

- Inhibitors reported--total 6--4 possible PTPs
 - 2 PUPs or < 20EDs
 - **4** confirmed 20-100 ED
- 345 million units distributed 1999-2003
 - assume annual dose of 212,000 IU per adult (full prophylaxis)
 - conservative estimate as most Koate DVI used in developing world where prophylaxis is not widespread in adults
 - ~ 1627 patient years of exposure
 - **Rate = 4/1627pt yrs = 2 per 1000 pt yrs**



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